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Low Counts of B Cells, Natural Killer Cells, Monocytes, Dendritic Cells, Basophils, and Eosinophils are Associated with Postengraftment Infections after Allogeneic Hematopoietic Cell Transplantation



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ABSTRACT

Hematopoietic cell transplant (HCT) recipients are immunocompromised and thus predisposed to infections. We set out to determine the deficiency of which immune cell subset(s) may predispose to postengraftment infections. We determined day 28, 56, 84, and 180 blood counts of multiple immune cell subsets in 219 allogeneic transplant recipients conditioned with busulfan, fludarabine, and Thymoglobulin. Deficiency of a subset was considered to be associated with infections if the low subset count was significantly associated with subsequent high infection rate per multivariate analysis in both discovery and validation cohorts. Low counts of monocytes (total and inflammatory) and basophils, and low IgA levels were associated with viral infections. Low plasmacytoid dendritic cell (PDC) counts were associated with bacterial infections. Low inflammatory monocyte counts were associated with fungal infections. Low counts of total and naive B cells, total and CD56^{high} natural killer (NK) cells, total and inflammatory monocytes, myeloid dendritic cells (MDCs), PDCs, basophils and eosinophils, and low levels of IgA were associated with any infections (due to any pathogen or presumed). In conclusion, deficiencies of B cells, NK cells, monocytes, MDCs, PDCs, basophils, eosinophils, and/or IgA plasma cells appear to predispose to postengraftment infections.

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INTRODUCTION

Hematologic malignancies and benign disorders of hematopoiesis are often cured with allogeneic hematopoietic cell transplantation (HCT). However, HCT recipients are immunocompromised because of multiple factors such as a relatively small number of immune cells transferred with the graft, limited differentiation of the grafted hematopoietic stem cells into some immune cell subsets like T cells, and pharmacologic immunosuppression. Therefore, HCT recipients are at a substantial risk of infections, causing morbidity and mortality. This immunocompromised state

lasts for at least 1 year [1–5]. Known predictive factors for high infection rates include an unrelated or HLA mismatched related donor (compared with a matched sib donor), cord blood or marrow graft (compared to mobilized blood stem cell graft), T cell–depleted graft, and the presence of significant graft-versus-host disease (GVHD) [5–7]. However, these known predictive factors cannot reliably distinguish between patients who will or will not develop infections. Thus, the discovery of new predictive markers is of interest.

Low count of an immune cell subset could be a useful marker for subsequent infections. More important, association between a low immune cell subset count and subsequent infections may help us understand the mechanism by which patients are rendered susceptible to infections. In addition, if it was known which immune cell subset deficiency predisposes to infections, the risk of infections

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could theoretically be minimized by the infusion of that subset from the donor, except if a high count of the subset could predispose to GVHD (eg, T cells) [8]. Whereas neutropenia appears to be the cardinal predisposing factor for early (pre-engraftment) infections, little is known about the deficiency of which immune cell subset(s) predisposes to postengraftment infections [9,10]. This knowledge is completely lacking for the setting of in vivo T cell depletion with polyclonal rabbit antithymocyte globulin, which has been increasingly used to prevent GVHD [11–15]. We set out to determine, in the setting of antithymocyte globulin-conditioned HCT, the low counts of which immune cell subsets on days 28, 56, 84, or 180 are associated with high rates of subsequent infections.

METHODS

Patients and Transplantation

Heparinized blood was collected from 231 patients who had undergone first allogeneic marrow or mobilized blood stem cell transplantation in Calgary between December 1, 2004 and August 31, 2010 and agreed to participate in this Research Ethics Board–approved study. Twelve patients were excluded because of graft failure, death, or relapse before day 30. The remaining 219 patients were divided into a discovery cohort (transplant date between December 1, 2004 and August 31, 2008) and a validation cohort (transplant date between September 1, 2008 and August 31, 2010) (Table 1).

Patients received myeloablative conditioning, typically fludarabine (250 mg/m² i.v.), busulfan (approximately 12.8 mg/kg i.v.), and antithymocyte globulin (4.5 mg/kg i.v.), and additional GVHD prophylaxis with methotrexate (days 1, 3, 6, and 11) and cyclosporine from day –1 until 3 to 6 months post-transplant or longer in the case of chronic GVHD [16]. Conditioning of some patients included total body irradiation (TBI) (4 Gy) [17].

Clinically significant GVHD (grades II to IV acute GVHD or chronic GVHD needing systemic therapy) was treated with a corticosteroid ± other immunosuppressive drug(s). Supportive care was similar for all patients. No antibacterial or antifungal prophylaxis was given routinely (except for trimethoprim-sulfamethoxazole for *Pneumocystis* prophylaxis). *Pneumocystis* prophylaxis was given until 6 months post-transplant or longer (in the case of chronic GVHD needing systemic therapy). Acyclovir was used until 6 to 12 months post-transplant or longer (in the case of chronic GVHD needing systemic therapy).

All blood products were from cytomegalovirus- (CMV) seronegative donors and were leukocyte depleted. CMV DNAemia was monitored; preemptive therapy was used [18]. Monitoring of Epstein-Barr virus DNAemia was not done, neither was monitoring for any other virus apart from CMV. Follow-up for infections ended at the time of death, graft failure, relapse, second malignancy, last contact, or day 730, whichever occurred first. Median follow-up for infections was 730 days (range, 34 to 730 days).

Immune Cell Subset Enumeration

The blood for enumeration of immune cell subsets was drawn on approximately days 28, 56, 84, and 180 after HCT (Table 1). The counts of most subsets were determined by flow cytometry as described [8] and defined as shown in Supplementary Table 1. Total WBC, eosinophil, and neutrophil counts were determined by a clinical hematology laboratory (Calgary Laboratory Services, Calgary, Alberta, Canada or other accredited laboratory). As a surrogate of total body plasma cell count and function, IgM, IgG, and IgA serum levels were measured by a clinical laboratory on days 84 and 180 post-transplant (typically using automated immunoturbidimetry analyzer, Integra 800; Roche Diagnostics, Mannheim, Germany). Patients who received IgG supplementation within 2 months before a time point were excluded from analysis of the association between IgG level on that time point and subsequent infections. The median counts of the cell subsets at the studied time points have been published [19] and for convenience are also given in Table 2.

Definitions of Infections

Definite infection was defined as an illness with symptoms and signs consistent with an infection and microbiologic documentation of a pathogen. For zoster virus, clinical diagnosis was considered sufficient. Microbiologic documentation included isolation of the pathogen by culture from a sterile site or a nonsterile site (if from a nonsterile site, the organism had to be clinically judged as pathogenic) or histologic/immunohistologic evidence. Culture-documented bacteremia or fungemia was counted even in the absence of symptoms or signs of infection, unless the organism was clinically judged as contaminant. CMV reactivation was counted as a

viral infection only if treated (preemptively or because of presumed or documented CMV disease) [18]. Post-transplant lymphoproliferative disorder was counted as a viral infection and was defined as an illness with signs or imaging results consistent with post-transplant lymphoproliferative disorder (eg, fever not due to other causes, lymphadenopathy, splenomegaly, or a mass) with Epstein-Barr virus DNA above 400 copies/μg leukocyte DNA or in situ hybridization/immunohistologic evidence of post-transplant lymphoproliferative disorder.

Presumed (clinical) infection was defined as illness with symptoms and signs consistent with an infection and no microbiologic documentation of a pathogen. However, presumed oral, gastrointestinal, conjunctival, and respiratory tract infections were discounted because they could not be reliably distinguished from GVHD or allergy. Fever without other symptoms/signs was also discounted because it could not be reliably attributed to an infection. Hemorrhagic cystitis was discounted because it could not be differentiated from conditioning regimen–induced cystitis. Sinusitis and pneumonia were counted only if radiologically documented.

A recurrent infection was counted as multiple infections if the episodes were separated by more than a 4-week asymptomatic period. A chronic infection (with asymptomatic periods lasting ≤4 weeks) was counted as 1 infection. A polymicrobial infection of 1 organ or several adjacent organs was counted as 1 infection. An infection in ≥2 nonadjacent organs because of the same microorganism was counted as 1 infection (disseminated).

Severe infection was defined as infection resulting in hospitalization. For infections occurring during hospitalization, severe infection was defined as infection that would result in hospitalization if the patient was an outpatient.

Infection rate was defined as the number of infections per 365 days at risk within a post-transplant time interval. Infection rates were assessed for any infections (definite or presumed), any severe infections (severe definite or severe presumed), severe definite infections, viral infections, bacterial infections, and fungal infections.

STATISTICS

Spearman rank correlation was used to evaluate correlations between the counts of each immune cell subset at predefined time points and the rate of infections in subsequent time intervals (eg, for day 84, intervals of days 84 to 179, 84 to 364, and 84 to 730 were considered; Supplementary Figure 2). For each immune cell subset for which the count appeared to be significantly associated with an infection rate in a subsequent time interval ($P < .05$), a multivariate analysis (MVA) was performed as Poisson regression, comparing infection rates in patients with high versus low subset counts. Cutoff between high and low counts of an immune cell subset was determined using receiver-operator characteristic curves as the point with the highest sum of specificity and sensitivity for developing at least 1 infection in the interval. In cases where the point of the highest sum of specificity and sensitivity fell outside of the 10th to 90th percentile, the point of the highest sum of specificity and sensitivity within the 10th to 90th percentile was used. The MVA was done to adjust for factors that could predispose to infections (other than the cell subset count). The covariates we included were significant GVHD (grades II to IV acute GVHD or chronic GVHD needing systemic therapy) in that interval (yes or no), donor type (HLA-matched sibling versus other), and stem cell source (marrow versus blood stem cells).

In the Poisson regression, days at risk were used as the offset. In the MVA, $P < .01$ was considered significant. This was an arbitrary correction for multiple comparisons; the correction was far less strict than Bonferroni correction because we assumed that counts of the various immune cell subsets were partly related to each other. For every association significant in the discovery cohort, the association was analyzed by MVA in the validation cohort. To be considered validated, the association must have been significant at $P < .05$ in the validation cohort. Additionally, the median percentile of the rate of infections in the low subset count group had to be equal or higher than that in the high subset

Table 1

Patient Characteristics

Characteristic	Discovery Cohort (n = 133)	Validation Cohort (n = 86)	Significance of Difference*
Median patient age, yr	47 (range, 19–66)	50 (range, 19–66)	.164
Median donor age, yr	35 (range, 15–67)	37 (range, 12–68)	.786
Patient sex	79 M, 54 F (59% M, 41% F)	50 M, 36 F (58% M, 42% F)	.853
Donor sex	89 M, 44 F (67% M, 33% F)	48 M, 38 F (56% M, 44% F)	.097
Diagnosis/disease stage at transplant [†]			.933
Poor risk	58 (44%)	38 (44%)	
Good risk	75 (56%)	48 (56%)	
Diagnosis			.007
AML in first remission	42 (32%)	27 (31%)	
AML beyond first remission [‡]	16 (12%)	10 (12%)	
ALL in first remission	10 (8%)	12 (14%)	
ALL beyond first remission	5 (4%)	5 (6%)	
CML in first chronic/accelerated phase	8 (6%)	0	
CML in blast or second chronic/accelerated phase	2 (~1%)	0	
CMML	5 (4%)	0	
CLL	2 (~1%)	7 (8%)	
Non-Hodgkin lymphoma	21 (16%)	7 (8%)	
Hodgkin lymphoma	2 (~1%)	0	
Myelodysplasia/myelofibrosis	14 (11%)	17 (20%)	
Aplastic anemia	4 (3%)	0	
Other [§]	2 (~1%)	1 (1%)	
Stem cell source			.020
Bone marrow	8 (6%)	0	
Blood stem cells	125 (94%)	86 (100%)	
Donor–recipient CMV serostatus at HCT			.687
Positive–positive	37 (28%)	28 (33%)	
Positive–negative	11 (8%)	7 (8%)	
Negative–positive	30 (23%)	19 (22%)	
Negative–negative	54 (41%)	27 (31%)	
Unknown or indeterminate	1 (~1%)	5 (6%)	
Donor–recipient EBV serostatus at HCT			.106
Positive–positive	117 (88%)	65 (76%)	
Positive–negative	4 (3%)	7 (8%)	
Negative–positive	9 (7%)	9 (10%)	
Negative–negative	0	1 (1%)	
Unknown or indeterminate	3 (2%)	4 (5%)	
Conditioning (in addition to antithymocyte globulin)			.058
Fludarabine + busulfan	44 (33%)	27 (31%)	
Fludarabine + busulfan + TBI	81 (61%)	59 (69%)	
Other	8 (6%)	0	
Donor type			.460
HLA-matched sibling	64 (48%)	37 (43%)	
Other [¶]	69 (52%)	49 (57%)	
Median day of engraftment [#]	14 (range, 10–27)	14 (10–24)	.537
Graft failure after day 30	1 (~1%)	0	.420
Acute GVHD by grade			.301
None	59 (44%)	33 (38%)	
Grade I	38 (29%)	32 (37%)	
Grade II	23 (17%)	12 (14%)	
Grade III	12 (9%)	5 (6%)	
Grade IV	1 (~1%)	2 (2%)	
Indeterminate	0	1 (1%)	
Chronic GVHD			.091
None	72 (54%)	44 (51%)	
Not needing systemic therapy	13 (10%)	15 (17%)	
Needing systemic therapy	48 (36%)	27 (32%)	
Relapse	26 (20%)	18 (21%)	.868
Second malignancy ^{**}	11 (8%)	5 (6%)	.495
Death	41 (31%)	27 (31%)	.929
Median follow-up (days post-transplant) ^{††}			
For infections	730 (range, 38–730)	730 (range, 34–730)	.385
For infections among patients without relapse/ graft failure/second malignancy/death by day 730	730 (range, 92–730)	730 (range, 68–730)	.895
For death	835 (range, 59–1657)	883 (range, 44–1448)	.374
For death among surviving patients	1018 (range, 181–1532)	1010 (range, 68–1448)	.569
Actual median blood draw days post-transplant			
Day 28	28 (range, 23–36)	27 (range, 24–34)	.025
Day 56	56 (range, 50–68)	55 (range, 51–64)	.172
Day 84	84 (range, 77–105)	83 (range, 75–96)	.029
Day 180	182 (range, 160–200)	181 (range, 159–207)	.860
Number of patients with immune cell subsets enumerated			
Day 28	113	81	Not applicable
Day 56	114	78	Not applicable

(Continued on next page)

Table 1
(continued)

Characteristic	Discovery Cohort (n = 133)	Validation Cohort (n = 86)	Significance of Difference*
Day 84	112	74	Not applicable
Day 180	74	57	Not applicable

AML indicates acute myeloid leukemia; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; CLL, chronic lymphocytic leukemia; EBV, Epstein-Barr virus.

* Significance of difference was determined by Mann-Whitney-Wilcoxon test for ordinal variables and by chi-square test for nominal variables.

† Good risk disease was defined as acute leukemia in first remission, CML in first chronic or accelerated phase, myelodysplasia with <5% marrow blasts, or aplastic anemia. All other diseases/disease stages were considered poor risk.

‡ Includes any AML that evolved from myelodysplasia or myelofibrosis.

§ Acute undifferentiated leukemia (n = 1, discovery cohort), unclassifiable myeloproliferative syndrome (n = 1, discovery cohort), and myeloproliferative syndrome (n = 1, validation cohort).

|| Combinations of cyclophosphamide, etoposide, melphalan, or TBI.

¶ 10/10- (HLA-A, -B, -C, -DRB1, -DQB1) allele matched unrelated donors (n = 48 discovery cohort, n = 40 validation cohort), 9/10-allele matched unrelated donors (n = 18 discovery, n = 7 validation), 8/10-allele matched unrelated donors (n = 2 discovery, n = 1 validation), 10/10-allele matched related nonsibling donors (n = 0 discovery, n = 1 validation), or 9/10- allele matched related donors (n = 1 discovery, n = 0 validation).

* The first of 3 consecutive days with absolute neutrophil count $\geq 5/\text{nL}$.

** Including post-transplant lymphoproliferative disorder. Excluding nonmelanoma skin cancer and carcinoma in situ.

†† Follow-up for infections was until relapse, graft failure second malignancy, death, or last contact, whichever occurred first. The reason for ending follow-up at relapse, graft failure, or second malignancy was that the treatment of relapse, graft failure, or second malignancy influences the risk of infections. Follow-up for death was until death or last contact, whichever occurred first.

count group; if equal, then the 75th percentile in the low subset group had to be higher (similar criteria would have applied to subsets for which a high count was associated with a high infection rate; however, such subsets were not found). Analysis was carried out using StataC, version 11 (StataCorp 2009; *Stata Statistical Software: Release 11*; College Station, TX).

For most subsets that were associated with infections in a specific time interval, the association was between the subset count and any infections or definite infections (and not

specifically viral, bacterial, or fungal infections). This was likely due to the limited power of the analysis for viral, bacterial, and fungal infections, because any infections or definite infections far outnumbered viral, bacterial, or fungal infections. Thus, for each subset associated with any infections or definite infections (in a time interval), we combined the discovery and validation cohorts to ascertain with a higher power (higher than in either discovery or validation cohorts alone) whether there was an association between the subset and viral, bacterial, or fungal infections. If yes (MVA $P < .05$), these results are preceded with a “?” symbol in [Supplementary Tables 2 through 6](#) (to indicate that the certainty of association is relatively low because it could not be validated in a separate validation cohort).

Table 2
Median Counts of Cell Subsets (Per μL Blood)*

Subset	Day 28	Day 56	Day 84	Day 180	Day 365 [†]
B cells	2.8	40.6	35.1	106.0	175.5
Naive B cells	1.1	35.6	30.7	92.9	156.6
Memory B cells, nonswitched	.5	1.4	1.2	2.3	1.9
Memory B cells, switched	.3	.7	1.0	2.7	4.4
CD4 T cells	21.7	78.3	89.8	113.8	216.0
Naive CD4 T cells	.1	.3	.5	2.6	10.8
Memory or effector CD4 T cells	21.5	76.8	88.0	100.8	169.6
CD8 T cells	33.7	287.2	248.7	298.8	271.1
Naive CD8 T cells	.4	1.0	1.0	1.9	4.2
Memory or effector CD8 T cells	32.6	276.5	246.2	297.1	271.1
CD4 ⁺ CD8 ⁺ T cells	11.0	23.3	23.1	32.3	27.5
CD4 ⁺ CD8 ⁺ T cells	2.8	11.6	20.5	23.8	7.5
Regulatory CD4 ⁺ T cells	.4	.8	1.7	2.8	5.0
NKT cells, invariant (iNKT cells)*	.6	.6	.8	.8	.8
NK cells	319.0	256.7	201.4	161.6	163.9
CD16 ⁺ CD56 ^{high} NK cells	46.5	16.3	13.3	7.6	6.8
CD16 ⁺ CD56 ^{low} NK cells	93.2	113.2	96.1	85.0	83.9
CD16 ^{high} CD56 ⁺ NK cells	22.4	34.4	36.2	22.9	2.0
Monocytes	589.4	336.5	398.2	363.0	396.7
Inflammatory monocytes	433.1	256.4	268.2	258.0	312.1
Resident monocytes	134.8	75.1	90.6	76.0	84.9
MDCs	2.0	1.3	3.6	4.1	4.7
PDCs	3.6	1.5	1.5	1.2	3.1
Basophils	19.9	8.1	5.6	3.6	23.1
Eosinophils	100.0	100.0	100.0	100.0	—
Neutrophils	3000.0	2600.0	2400.0	2700.0	3300.0
WBCs	4500.0	4700.0	4300.0	4900.0	5400.0

NKT indicates natural killer T cells —, not recorded.

* Discovery and validation cohorts combined.

† Median counts for the day 365 timepoint for NKT cells are only for discovery cohort; validation cohort counts were not measured.

RESULTS

Infection Rates

A total of 535 infections (definite and presumed) occurred between days 28 and 730 in the 219 patients (in discovery and validation cohorts combined) over 111,188 days at risk. The average rate of any (definite or presumed) infection was 8.01 per 365 days at risk between days 28 and 55, 4.51 per 365 days at risk between days 56 and 83, 2.27 per 365 days at risk between days 84 and 179, 1.22 per 365 days at risk between days 180 and 364, and .86 per 365 days at risk between days 365 and 730 ([Table 3](#) and [Supplementary Figure 2](#)). Analogous rates of definite infections, viral infections, bacterial infections, fungal infections, any severe infections, and severe definite infections are shown in [Table 3](#) and [Supplementary Figure 2](#).

To determine which clinical/demographic factors influenced the risk for infections in our population, we performed an MVA to determine the association between patient age (<45 versus ≥ 45 years), matched sibling versus other donor, HLA match (10/10 matched versus $\leq 9/10$ matched), graft type (marrow versus peripheral blood stem cells), significant GVHD (at any time), and infection rates in the longest intervals days 28 to 364 and 56 to 730. We found that only significant GVHD at any time was significantly associated with infections (see [Supplementary Figure 5](#)).

Lymphocyte Subsets Associated with Infections

For total B cells, low day 180 counts were associated with a high rate of any infections between days 180 and

Table 3
Rates of Infections within Time Intervals

Time Intervals		Any	Definite	Viral	Bacterial	Fungal	Any Severe	Severe Definite
One interval	Days 28-55	8.01	7.53	6.07	1.27	.24	4.55	4.37
	Days 56-83	4.51	4.19	2.58	1.42	.45	2.77	2.64
	Days 84-179	2.27	2.02	1.18	.61	.24	.92	.84
	Days 180-364	1.22	.99	.59	.29	.12	.41	.37
	Days 365-730	.86	.67	.33	.24	.12	.35	.31
Two intervals	Days 28-83	6.31	5.91	4.38	1.34	.34	3.69	3.53
	Days 56-179	2.81	2.54	1.52	.81	.29	1.36	1.27
	Days 84-364	1.61	1.37	.81	.41	.17	.60	.55
	Days 180-730	.99	.79	.42	.26	.12	.37	.34
Three intervals	Days 28-179	3.87	3.56	2.45	.90	.28	2.01	1.90
	Days 56-364	1.91	1.67	1.00	.51	.20	.83	.77
	Days 84-730	1.22	1.01	.56	.32	.14	.47	.43
Four intervals	Days 28-364	2.52	2.26	1.51	.59	.20	1.20	1.13
	Days 56-730	1.40	1.18	.67	.38	.16	.59	.54

Average rates of any infections (definite or presumed), definite infections, viral infections, bacterial infections, fungal infections, any severe infections and severe definite infections per 365 days at risk are given for the time intervals for which statistical analyses were carried out. The top 5 intervals are the basic (short) intervals ("1 interval"). The following 4 intervals are longer, each consisting of 2 neighboring basic intervals ("2 intervals"). The following 3 intervals are even longer, each consisting of 3 neighboring basic intervals ("3 intervals"). The following 2 intervals are the longest, each combining 4 neighboring basic intervals ("4 intervals"). Discovery and validation cohorts were combined for this analysis.

364 (significant in both discovery and validation cohorts) (Supplementary Table 2 and Supplementary Figure 3). This association may have been primarily due to the association between low counts of total B cells and viral infections (significant in combined discovery and validation cohort). For naive B cells, which constitute most blood B cells in both healthy and post-transplant individuals [19], low counts were associated with high rates of any, definite, any severe, and severe definite infections. Both viral and bacterial infections appeared to be increased in the patients with low naive B cell counts. For memory B cells, no association with an infection rate was found. For CD4 and CD8 T cells and their subsets and for invariant natural killer (NK) T cells, no association with an infection rate was found.

For total NK cells, low day 56 counts were significantly associated with high rates of any infections in the day 56 to 364 time interval (Supplementary Figure 3). Both viral and bacterial infections appeared to be increased in the patients with low total NK cell counts. For CD16⁺CD56^{high} NK cells, which constitute a minority subset of blood NK cells in both healthy and post-transplant individuals [19], low day 56 counts were associated with high rates of both any and definite infections and appeared to be associated with high rates of viral, bacterial, and fungal infections. For

CD16⁺CD56^{low} NK cells, which is the most numerous subset of circulating NK cells [19], no association with an infection rate was found.

Monocytes/Monocyte Subsets Associated with Infections

Inflammatory monocytes are the majority subset and resident monocytes the minority subset of monocytes in both healthy and post-transplant individuals (Supplementary Table 3) [19]. Low counts of both total and inflammatory monocytes on day 56 were associated with high rates of any (Supplementary Figure 3), definite, viral (Figures 1 and 2), and, possibly, bacterial infections. Also, low counts of total monocytes on day 56 were associated with high rates of severe and severe definite infections. Low counts of inflammatory monocytes on day 56 were associated with high rates of fungal infections. Additionally, on day 84 inflammatory monocytes were found to be possibly associated with viral, bacterial, and fungal infections. For resident monocytes, no association with an infection rate was found.

Dendritic Cell Subsets Associated with Infections

Low myeloid dendritic cell (MDC) counts on days 28, 56, and 84 were associated with high rates of any (Supplementary Table 4 and Supplementary Figure 3),

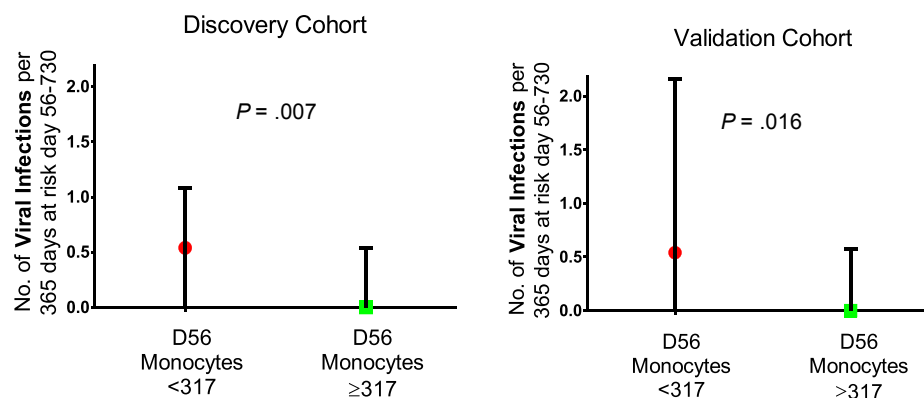


Figure 1. Low monocyte count on day 56 associated with subsequent viral infections. Rates of day 56 to 730 viral infections per 365 days at risk in patients with day 56 monocyte counts above and below receiver-operator characteristic determined cutoff are displayed. Shown are the medians and interquartile ranges. Cutoff monocyte counts are given in number of cells per μL .

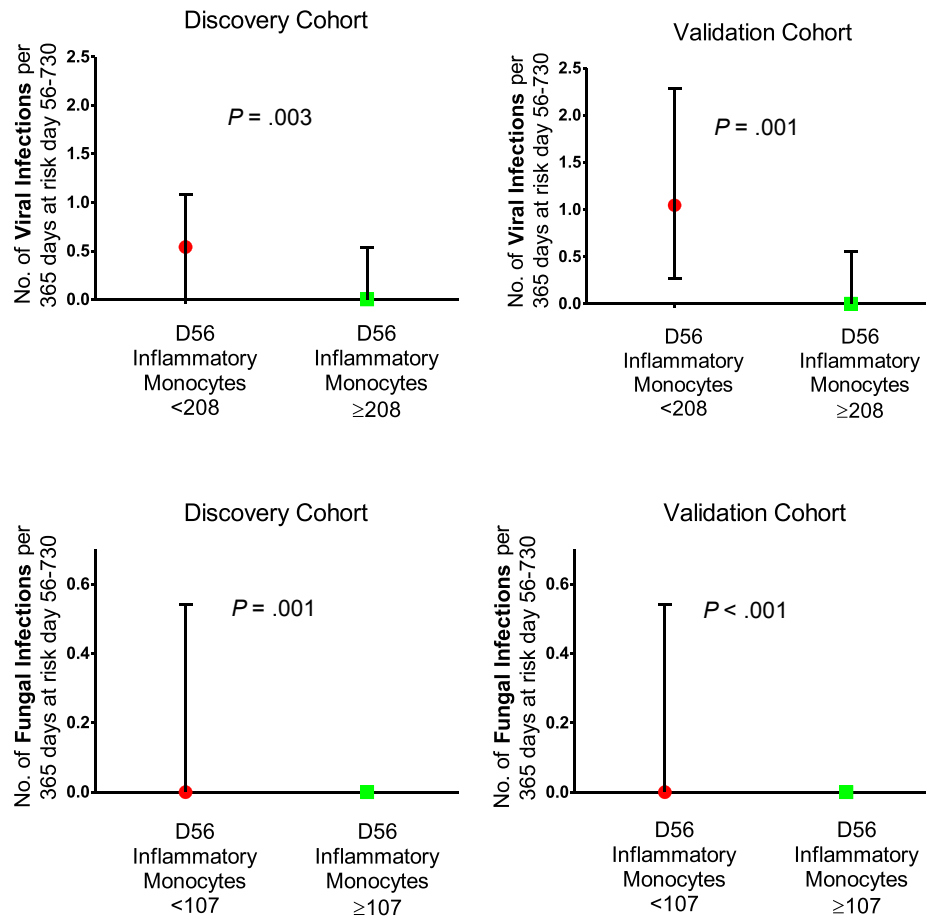


Figure 2. Low inflammatory monocyte count on day 56 associated subsequent viral and fungal infections. Rates of day 56 to 730 viral and fungal infections per 365 days at risk in patients with day 56 inflammatory monocyte counts above and below receiver-operator characteristic determined cutoff are displayed. Shown are the medians and interquartile ranges. Cutoff inflammatory monocyte counts are given in number of cells per μL .

definite, or any severe infections. The counts also appeared to be associated with viral, bacterial, and fungal infections.

Low plasmacytoid dendritic cell (PDC) counts on day 56 were associated with high rates of any (Supplementary Figure 3) and bacterial infections (Figure 3) and, possibly, viral and fungal infections. Low PDC counts on day 84 were associated with definite and severe definite infections and, possibly, viral, bacterial, and fungal infections.

Granulocyte Subsets Associated with Infections

Low basophil counts on day 56 were associated with high rates of definite (Supplementary Figure 3) and viral infections (Figure 4). Low basophil counts on day 84 were associated with high rates of definite and any severe infections (Supplementary Table 5).

Low eosinophil counts on day 56 were associated with high rates of any (Supplementary Figure 3) and definite

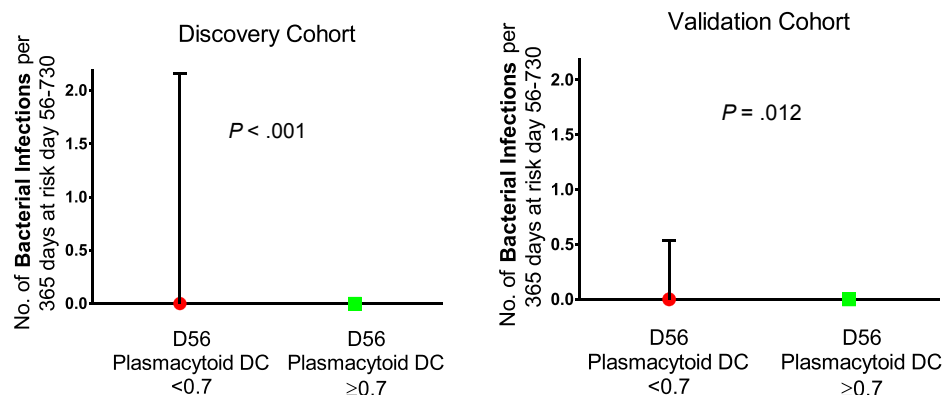


Figure 3. Low PDC count on day 56 associated with subsequent bacterial infections. Rates of day 56 to 730 bacterial infections per 365 days at risk in patients with day 56 PDC counts above and below receiver-operator characteristic determined cutoff are displayed. Shown are the medians and interquartile ranges. Cutoff PDC count is given in number of cells per μL .

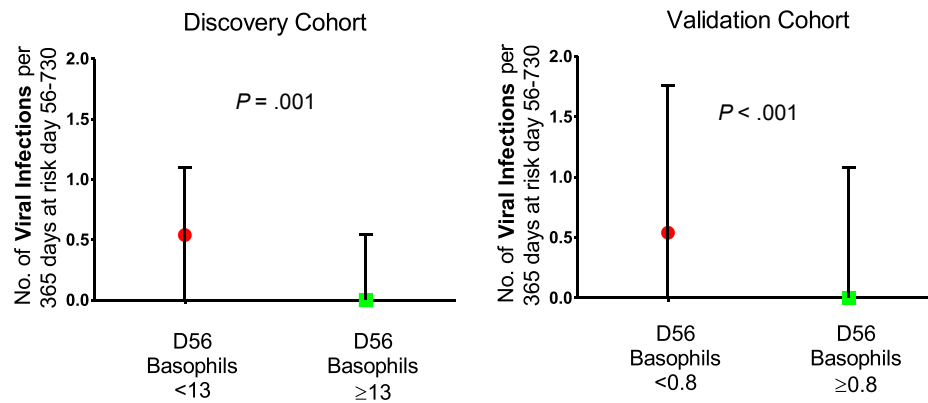


Figure 4. Low basophil count on day 56 associated with subsequent viral infections. Rates of day 56 to 730 viral infections per 365 days at risk in patients with day 56 basophil counts above and below receiver-operator characteristic determined cutoff are displayed. Shown are the medians and interquartile ranges. Cutoff basophil count is given in number of cells per μL .

infections and, possibly, viral and bacterial infections. There was no association between neutrophil counts on days 28, 56, 84, or 180 and an infection rate.

WBC Counts and Infections

There was no association between total WBC counts on days 28, 56, 84, or 180 and an infection rate.

Immunoglobulin Serum Levels Associated with Infections

Low IgA levels on day 84 were associated with high rates of any (Supplementary Figure 3), definite, and viral infections. There was no association between IgM or IgG levels on day 84 or 180 and an infection rate (Supplementary Table 6).

Correlations among Subsets

A limitation of our study is that if low counts of several subsets at 1 time point were associated with infections, it was impossible to determine whether deficiency of all subsets predisposed to infections versus whether deficiency of only 1 or a few of the subsets predisposed to infections (where low counts of the remaining subsets were only surrogates for the low counts of the subsets predisposing to infections). This problem did not pertain to day 28, because only 1 subset on day 28 was associated with infections (MDCs). This problem also did not pertain to day 180, because only 2 related subsets (B cells and IgA levels as surrogates for IgA plasma cells) were associated with infections. There was a correlation between day 180 B cell counts and IgA levels (Spearman rank correlation coefficient $r = .46$, $P = .001$), which is expected because B cells are expected to differentiate into IgA plasma cells. However, this problem pertained only to the day 56 and 84 time points.

On day 56, there was a significant correlation among all subsets associated with any or definite infections (ie, total and CD56^{high} NK cells, total and inflammatory monocytes, MDCs, PDCs, basophils, and eosinophils) (Supplementary Table 7). However, the correlation coefficients were relatively low (.16 to .67, apart from the expected association between total and inflammatory monocytes), suggesting an independent contribution of each subset to the predisposition to infections. Similarly, on day 84 there was a relatively weak (correlation coefficients .06 to .65) but significant correlation among most subsets associated with any or definite infections (Supplementary Table 8). Nevertheless,

there was no correlation between naive B cells and inflammatory monocytes, between basophils and inflammatory monocytes, between naive B cells and IgA, between inflammatory monocytes and IgA, or between MDCs and IgA. This suggested independent contribution to predisposition to infections of the subsets in these pairs of subsets.

Cell Subset Count in Patients with High and Low Infection Rates

To further display the relationship between subset counts and infection rates (beyond that shown in Table 3 and Supplementary Figures 2 and 3), we show the kinetics of reconstitution of all significant cell subsets (subsets associated with infections) in patients with high versus low infection rates (Supplementary Figure 4).

DISCUSSION

We discovered and validated associations between low counts of several immune cell subsets and high rates of postengraftment infections. The subsets that were associated and thus appear to predispose to the infections include B cells (total and naive), NK cells (total and CD16[−]CD56^{high}), monocytes (total and inflammatory), MDCs, PDCs, basophils, eosinophils, and IgA plasma cells.

Low B cells, especially the naive subset, appeared to predispose to any, definite, any severe, severe definite, and, probably, viral and bacterial infections (Supplementary Table 2), and low IgA levels appeared to predispose to any, definite, any severe, and viral infections (Supplementary Table 6 and Figure 5). Naive B cells should, after encountering their cognate antigen and appropriate costimulation, differentiate into memory B cells and plasma cells. It may be that with more naive B cells present on or after day 84, there is a higher number of B cells prepared to detect various pathogens and differentiate into the unique plasma cells, producing antibodies needed to curtail the pathogens via neutralization, complement-mediated lysis, or opsonized phagocytosis. Consistent with that, on post-hoc analysis there was a correlation or a trend towards a correlation between day 84 naive B cell counts and day 84 IgM, IgG, and IgA levels ($r = .21$, $.29$, and $.17$; $P = .016$, $.001$, and $.056$, respectively), and low day 84 IgA levels were associated with subsequent infections (Supplementary Table 6). Also, there was a correlation between naive B cell counts on day 84 and IgM, IgG, and IgA levels on day 180 ($r = .24$, $.25$, and $.31$;

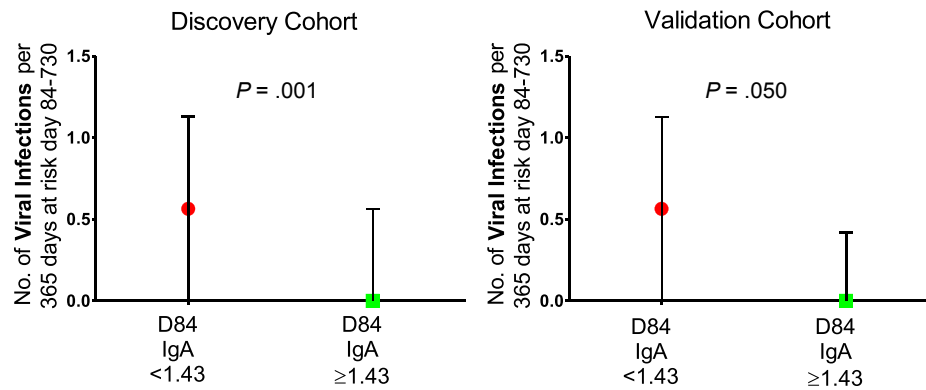


Figure 5. Low IgA level on day 84 associated with subsequent viral infections. Rates of day 84 to 730 viral infections per 365 days at risk in patients with day 84 IgA levels above and below receiver-operator characteristic determined cutoff are displayed. Shown are the medians and interquartile ranges. Cutoff IgA level is given in g/L.

$P = .012$, $.011$, and $.001$ respectively). Additionally, there was a correlation between day 180 B cell counts and day 180 IgA levels ($r = .46$, $P = .001$), and low day 180 IgA levels were associated with subsequent infections (Supplementary Table 6).

NK cells in general contribute to defense against infected cells without need for prior exposure [20]. $CD16^+CD56^{low}$ NK cells kill virus-infected cells by perforin or granzyme-mediated cytotoxicity. However, low counts of this subset were not associated with any or viral infections (Supplementary Table 2). It was the $CD16^-CD56^{high}$ NK cell subset, low counts of which were associated with any and probably viral, bacterial, and fungal infections (Supplementary Table 2). The $CD16^-CD56^{high}$ NK cells play less of a cytotoxic role but instead secrete large amounts of cytokines including $IFN-\gamma$ in response to IL-12 and IL-15, the latter being produced by monocytes and dendritic cells [21,22]. $IFN-\gamma$ activates monocytes [23]. Thus, the following positive feedback loop may be important for curtailing infections: Monocytes and dendritic cells secrete IL-12 and IL-15, which stimulate $CD56^{high}$ NK cells, which in turn secrete $IFN-\gamma$, which results in positive feedback stimulation of monocytes and dendritic cells [24]. Consistent with this hypothesis, there was a correlation between day 56 $CD56^{high}$ NK cell counts and counts of monocytes, MDCs, and PDCs (Supplementary Tables 7 and 8). Additionally, the monocytes, MDCs, and PDCs were also associated with infections (Supplementary Tables 2, 3, and 4).

We also note an important anomaly of $CD56^{high}$ NK cells: As opposed to all the other immune cells subsets that we enumerated, $CD56^{high}$ NK cells are abundant between 1 and 6 months post-transplant (higher counts than in healthy individuals) [19,25]. Thus, where an association between low counts of others subsets and infections can be interpreted as “deficiency of the subset predisposes to the infections,” an association between low $CD56^{high}$ NK cell counts and infections needs to be interpreted as *relative* deficiency of $CD56^{high}$ NK cells (fewer cells than what a post-transplant patient but not a healthy individual should ideally have) predisposes to the infections.

Low monocytes were also associated with infections, particularly viral infections (Supplementary Table 3, Figures 1 and 2). This is consistent with a previous study that showed low monocyte counts on day 80 to be associated with an increased rate of subsequent any and viral infections [26]. Monocytes are known for their role in early defense.

They are rapidly recruited to the site of infection and differentiate into macrophages and dendritic cells that stimulate adaptive immunity [27,28]. The infections were associated with low counts of inflammatory but not resident monocytes, possibly because inflammatory monocytes are particularly efficient in chemotaxis, phagocytosis, and antigen presentation [29]. In our study the inflammatory subset was associated with fungal infections, which supports previous evidence that inflammatory monocytes orchestrate antifungal immunity in the lungs [30]. Specifically, this may occur through the facilitation of CD4 T cells responses mediated by this inflammatory subset of monocytes [31]. Our finding of an association between total or inflammatory monocyte counts and viral or fungal infections suggests that monocytes play an important role in antiviral and antifungal immunity of transplant recipients.

Dendritic cells initiate T cell mediated immune responses through the presentation of the HLA–peptide complex. PDCs produce type I interferons ($IFN-\alpha$ and $IFN-\beta$) in response to toll-like receptor–virus engagement [32]. These interferons in turn block viral replication. Our observation of the association between low PDCs and primarily bacterial infections (Supplementary Table 6 and Figure 3) suggests that PDCs may play a role in not only antiviral but also antibacterial defense. MDCs stimulate T cells, in part through the secretion of IL-12, which also stimulates NK cells [33]. Per our results, MDCs appear to play a role in defense against viruses, bacteria, and fungi (Supplementary Table 6).

Basophil counts were associated with any, definite, any severe, and viral infections (Supplementary Table 5 and Figure 4). Basophils are largely known for their role in immediate hypersensitivity reactions [34,35]. However, basophils have been found to also play a role in adaptive humoral immunity [36,37]. Secretion of IgM or a class switch to IgG or IgA by B cells is enhanced by activated basophils [36]. This is likely mediated through production of IL-4 and IL-6 by the basophils [37]. Therefore, the protective role of basophils may be via B cell activation/stimulation of antibody production. As a side question, post-hoc we wished to evaluate whether basophil counts determined by the clinical hematology laboratory were also associated with infections (as were basophil counts determined by flow cytometry). There was no significant association between the clinical hematology laboratory–determined basophil counts and infections (data not shown). We speculate that the discrepancy may be due to the higher accuracy of basophil count

determination by flow cytometry versus by a clinical hematology laboratory (typically using an automated 5-part differential analyzer and, in case of flagged results, manual differential).

Eosinophil counts were associated with any and definite infections and, possibly, bacterial and viral infections (Supplementary Table 5). Although their complete function is not yet fully understood, eosinophils are widely known to participate in responses to allergens and helminthes. However, studies have shown an antiviral effect as well, specifically for respiratory viruses [38–40]. Viral activation of eosinophils may cause their degranulation, which may target the virus or virus-infected cells or may cause resistance of cells to viral infection [38]. It is also possible that the presence of a virus stimulates eosinophils to release Th1, Th2, and regulatory cytokines and chemokines [38,41].

Our study did not find any associations between CD4 T cells, CD8 T cells, or total T cells and infections, which has previously been reported in numerous studies [42–47]. This lack of associations could be due to the following reasons: The studies that included patients with GVHD but did not include GVHD as a covariate may have had positive findings because immunosuppressive therapy used for the treatment of GVHD may have altered (lowered) the T cell count. In our MVA we included GVHD as a covariate. Additionally, we may have had insufficient power to detect the existing association between low T cell counts and infections.

Given the use of the discovery to validation cohort design, we can be reasonably confident in our positive findings. However, a disadvantage of our discovery to validation cohort design is that it has a relatively limited power to detect weak associations. Thus, in our study a lack of association between a low immune cell subset count and infections should not be interpreted as “deficiency of the subset plays no role in predisposition to infections.” Another reason this assumption would be incorrect is the fact that we measured the counts of the subsets in the peripheral blood and not in tissues, where their counts may differ and presence may be more relevant.

Another limitation of our study is our inability to isolate the individual contribution of immune cell subsets to the association with any or definite infections (Supplementary Tables 7 and 8). However, this problem pertained only to days 56 and 84 and not days 28 and 180. Moreover, even for days 56 and 84 the correlation coefficients between different immune cell subset counts were relatively low, suggesting independent contribution of all or most subsets to the predisposition to infections in general. We considered adding the “competing” subsets as covariates into the MVA; however, this was not possible because our sample size was too small for MVA with more than 3 covariates. Additionally, with regards to this study limitation, we believe it is more informative to focus on identifying cell subsets that predispose to a class of pathogens (viruses, bacteria, fungi) rather than infections in general, as preemptive therapy in the future would be class-specific (eg, a future broad-spectrum antiviral drug for viruses, a broad-spectrum antibacterial for bacteria, a broad-spectrum antifungal for fungi). For most class-specific infections our results are informative: For day 56 subsets and viral infections, only inflammatory monocytes and basophils were associated with viral infections (apart from total monocytes, which are composed mostly of inflammatory monocytes). Because the correlation between day 56 counts of inflammatory monocytes and basophils was weak ($r = .26$), deficiencies of both inflammatory monocytes

and basophils likely contribute to the predisposition to viral infections.

We also acknowledge the frequent use of TBI at our center may limit the generalizability of our findings to patients at centers that use TBI less frequently. It is possible that TBI could have killed recipient T cells that contribute to antiviral immunity after HCT [48] or recipient macrophages or dendritic cells, which could lead to impaired antigen presentation to donor T cells.

We conclude that deficiency of B cells (primarily naive), NK cells (primarily CD56^{high}), monocytes (primarily inflammatory), MDCs, PDCs, basophils, and/or eosinophils may predispose to postengraftment infections. For viral infections, deficiencies of monocytes, basophils, and IgA plasma cells appear to be the predisposing factors. For bacterial infections, PDC deficiency appears to be the predisposing factor. For fungal infections, inflammatory monocyte deficiency appears to be the predisposing factor. Fortunately, in a previous study we did not find high counts of any 1 of these subsets to be associated with an increased likelihood of developing GVHD, relapse, or overall mortality [8]. Thus, it could be studied whether infusion of these subsets (harvested from HCT donors) could prevent postengraftment infections. Studies of infusion of donor CD56^{high} NK cells, monocytes, PDCs, or basophils would be of particular interest, because high counts of CD56^{high} NK cells, total and inflammatory monocytes, PDCs, and basophils in the first 3 months after HCT appear to protect against the development of chronic GVHD [8]. Thus, both chronic GVHD and postengraftment infections could theoretically be prevented.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.bbmt.2015.09.003>.

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